DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO FILE
BNF42
September 12, 1997

Subject: Ringspot virus resistant papaya line 55-1

Keywords: papaya, *Carica papaya,* papaya ringspot virus (PRSV) coat protein, kanamycin resistance (*kan*^{r}) gene, aminoglycoside 3' phosphotransferase II (APH(3') II).

Introduction

Dr. Richard Manshardt of the University of Hawaii and Dr. Dennis Gonsalves of Cornell University initially consulted with FDA regarding this product in December 1994. On January 3, 1997, they submitted a safety and nutritional assessment of their transgenic virus resistant papaya line 55-1, followed by additional information regarding the safety and nutritional assessment of their papaya line 55-1 on July 25, 1997.

Intended Effect of the Genetic Modification

The red-pigmented "Sunset" papaya was modified to contain the coat protein gene of the papaya ringspot virus (PRSV), thereby conferring the new transgenic papaya line 55-1 with the ability to resist the virus.

Molecular Characterization and Alteration

An *Agrobacterium tumefaciens*-binary plasmid, which contained the PRSV coat protein, the β-glucuronidase (GUS) gene and the kanamycin resistance (*kan*^{r}) gene under the control of plant promoters within the T-DNA borders of the plasmid, was used in the development of the transgenic papaya line 55-1. Outside the T-DNA borders, the plasmid contained two genes which confer resistance to the antibiotics, gentamycin and tetracycline, under control of bacterial promoters. According to the developers of the transgenic papaya, the plasmid DNA was incorporated into the papaya genome by particle bombardment.

To determine which genes were transferred into the papaya genome, the papaya developers performed Southern blot analyses using genomic DNA from the progeny of the papaya line 55-1 crossed with a non-transgenic "Sunset" papaya. Three probes were used to detect DNA from inside the T-DNA border. A probe for each of the three genes, PRSV coat protein, GUS, and *kan*^{r}, hybridized to DNA sequences in the 55-1 papaya genome. Therefore, according to the papaya developers, the PRSV coat protein, GUS and *kan*^{r} genes were incorporated into the 55-1 papaya genome. Three probes, which are homologous to DNA sequences within the antibiotic resistance markers, were used to detect DNA from outside the

T-DNA borders. One probe hybridizes to the gentamycin resistance gene and two probes (ORIV-TET and ORIB-TET) hybridize to two different regions of the tetracycline resistance gene. Southern blots indicate that some DNA from outside the T-DNA borders was incorporated into the 55-1 papaya genome. For papaya line 55-1, hybridization is not observed with the probes to the gentamycin resistance gene or ORIV-TET DNA sequences but is observed with the probe to the ORIB-TET DNA sequences. Therefore, the papaya developers concluded that line 55-1 contains only part of the tetracycline resistance gene and does not contain the gentamycin resistance gene.

Regulatory Considerations

The safe use of pesticidal substances and the use of selectable markers as pesticidal inert ingredients in the development of virus resistant plant varieties are under the regulatory purview of the Environmental Protection Agency (EPA). EPA regulates the use of the introduced genetic material encoding the papaya ringspot viral coat protein and the selectable marker (including associated sequences required for expression) as well as the expression product. Therefore, although the papaya developers provided information regarding the safety of the ringspot viral coat protein and APH(3') II when expressed in papaya, we have not addressed the safe use of papaya ringspot viral coat protein as a pesticide or the safe use of APH(3') II as a pesticidal inert ingredient. We have, however, considered possible alterations in levels of nutrients and natural toxicants that may have occurred in papaya line 55-1 as a result of the genomic modification.

Compositional Analysis

Endogenous toxicants

The papaya developers noted that there was a report of a significant association between frequent occurrence of papaya in the diet and incidence of prostate cancer in Japanese men more than 70 years of age, but it was not clear whether this association had any biological significance or was merely due to chance or confounding factors. This trend was not significant in other ethnic groups studied. Also, the papaya developers noted that newspaper reports have stated that eating papaya may induce abortion in pregnant women. According to the papaya developers, scientific studies indicate that these reports are connected with the presence of benzyl isothiocyanate (BITC) which is associated with latex in green papaya tissues, but ripe papaya fruits lack latex and have virtually no BITC content. Thus, the papaya developers conclude that because ripe papaya fruit lacks latex and has virtually no BITC content, oral consumption of ripe papaya fruit would have no effect as an abortifacient.

The papaya developers analyzed fruit extracts by gas chromatography to determine the BITC content of the transgenic papaya and three varieties of non-transgenic papaya. BITC levels were measured in extracts from three immature fruits and three ripe fruits of each line/cultivar. The level of BITC in ripe fruit was much less than in immature fruit. The papaya developers report no significant differences in BITC content between transgenic and non-transgenic papayas from either immature or ripe fruit. They note that isothiocyanate concentrations in ripe papaya fruits are ten to 100 times lower than values of isothiocyanates reported in other foods, such as *Brassica* crops which include cruciferous vegetables such as broccoli, Brussels sprouts and cabbage. The papaya developers concluded that these facts indicate that BITC in ripe transgenic papaya fruits poses no special threat to human health.

Nutrients

The papaya developers referenced general literature values for a variety of nutrient parameters in their submission and claim that significant nutrient components occurring in papaya include sugar, vitamin A and vitamin C. Also, the papaya developers claim that in certain regions of the world the papaya makes an important contribution to the human diet, particularly as a source of vitamins A and C. Therefore, the papaya developers analyzed for these three nutrients, vitamin A, vitamin C, and total soluble solids (TSS), which is a measure of sugar content, in their transgenic plants.

The papaya developers conducted compositional analyses on the fruit of their transgenic papaya and control papaya plants. The analyses for total soluble solids (TSS) were performed using a number of fruits. With plants grown in PRSV-infested locations, the papaya developers observed a slightly higher TSS level for the transgenic fruits than PRSV-infected non-transgenic parental fruits, but a similar TSS content between transgenic fruits and non-transgenic PRSV-infected fruits of a commercial variety Kapoho. A similar TSS content was also observed between the transgenic fruits and healthy, thus devoid of PRSV infection, non-transgenic parental fruits.

For vitamin A and vitamin C, one set of data was obtained from frozen samples of ripe papaya fruit. Three fruits were combined to produce one sample for each line. The vitamin A and vitamin C content was determined for three transgenic lines, including line 55-1 and an F1 progeny from a cross between line 55-1 and a Kapoho cultivar, and four non-transgenic papaya lines/cultivars. For vitamin A only, a second set of data was obtained using ripe papayas from two transgenic papaya lines, including line 55-1, and six non-transgenic papaya lines.

The papaya developers state that the amount of vitamin A in the transgenic line 55-1 was comparable to other red-pigmented varieties of papaya analyzed and was within the range of values obtained for several commercial varieties of papaya. The amount of vitamin C observed was slightly lower for the transgenic line 55-1 compared to the non-transgenic parental lines. Because a number of factors, including ripeness of fruits and growing conditions, can affect vitamin content in fruits, variability has been observed and reported for vitamin levels in fruits. The amount of vitamin C in the transgenic papaya line 55-1 represents a high level of vitamin C for a food product (49.4-53.6 mg/100g, 82-89% DV) and is within the range observed in the literature for papaya.

Conclusions

The developers of the transgenic papaya have concluded, in essence, that the PRSV resistant papaya they have developed is not materially different, in terms of food safety and nutritional profile, from red-pigmented papaya varieties with a history of safe use. At this time, based on the papaya developers' description of ~~its~~ their data and analyses, the agency considers the consultation on the virus resistant transgenic papaya line, 55-1, to be complete.

Wendy Dixon, Ph.D.